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LEUKOCYTES MEDIATE ACID ASPIRATION INDUCED MULTI-ORGAN INJURY

BY

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Acid aspiration leads to lung injury characterized by neutrophil (PMN) sequestration and edema. This study investigates whether localized acid aspiration triggers both local and remote PMN sequestration and whether these cells are responsible for pulmonary edema and systemic organ injury. Rats pretreated with IV saline or rendered neutropenic underwent tracheostomy and insertion of a cannula into a lung segment. This was followed by instillation of either 0.1 N HCl or saline. After 30 min leukopenia was noted and circulating PMN produced H2O2. These activated cells were also primed to a

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second oxidative stimulus. In addition, 30 min after aspiration PMN were noted to be progressively sequestered in the non-aspirated lung as well as in the heart and kidney. This preceded the increased permeability and edema that developed in the lungs and systemic organs. At 3 h a correlation was found between the sequestered PMN and the increase in wet/dry ratio (W/d) of the non-aspirated lung ($r = 0.82$, $p < 0.05$). In neutropenic rats there was a significant ($p < 0.05$) reduction of localized aspiration induced: plasma thromboxane B2 and leukotriene B4 synthesis; generalized increase in lung W/d; and protein level in bronchoalveolar lavage of the aspirated and non-aspirated lungs. Neutropenia also lowered aspiration induced myeloperoxidase activity in the heart and kidney along with W/d of these organs (all $p < 0.05$) These data indicate that localized acid aspiration activates circulating neutrophils and promotes their sequestration in the lungs and systemic organs. These cells are largely responsible for the multi-system organ injury.

ABSTRACT

Acid aspiration leads to lung injury characterized by neutrophil (PMN) sequestration and edema. This study investigates whether localized acid aspiration triggers both local and remote PMN sequestration and whether these cells are responsible for pulmonary edema and systemic organ injury. Rats pretreated with IV saline or rendered neutropenic underwent tracheostomy and insertion of a cannula into a lung segment. This was followed by instillation of either 0.1 N HCl or saline. After 30 min leukopenia was noted and circulating PMN produced H_2O_2. These activated cells were also primed to a second oxidative stimulus. In addition, 30 min after aspiration PMN were noted to be progressively sequestered in the non-aspirated lung as well as in the heart and kidney. This preceded the increased permeability and edema that developed in the lungs and systemic organs. At 3 h a correlation was found between the sequestered PMN and the increase in wet/dry weight ratio (W/d) of the non-aspirated lung ($r=0.82$, $p < 0.05$). In neutropenic rats there was a significant ($p < 0.05$) reduction of localized aspiration induced: plasma thromboxane B_2 and leukotriene B_4 synthesis; generalized increase in lung W/d; and protein level in bronchoalveolar lavage of the aspirated and non-aspirated lungs. Neutropenia also lowered aspiration induced myeloperoxidase activity in the heart and kidney along with W/d of these organs (all $p < 0.05$). These data indicate that localized acid aspiration activates circulating neutrophils and promotes their sequestration in the lungs and systemic organs. These cells are largely responsible for the multi-system organ injury.

INTRODUCTION

Acid aspiration induces a delayed pulmonary injury in the local area aspirated. This is associated with activation of circulating neutrophils to synthesize thromboxane (Tx) 2 hours after aspiration (30). Soon thereafter, there is alveolar infiltration of neutrophils and increase in microvascular permeability. The sequence of events following acid aspiration suggests a Tx and neutrophil dependent process. Thus, leukosequestration and lung injury were attenuated by treatment with a Tx inhibitor (8). However, interpretation of this observation is clouded by the possibility that the protection afforded by Tx inhibition could be due to avoidance of a direct vasotoxic effect of Tx on the pulmonary microcirculation in distinction to Tx acting as an autocoid to further activate PMN or to serve as a cofactor in the neutrophil-endothelial interaction (28).

Sequestered neutrophils mediate microvascular barrier dysfunction in other settings of the adult respiratory distress syndrome. Thus, an association has been found between PMN entrapment in the pulmonary microcirculation and the permeability increase following exposure to high oxygen tensions, ischemia and reperfusion, pancreatitis as well as microembolization. In these conditions neutrophil depletion has been found to attenuate lung edema (16,29,11,21). That the process of neutrophil microvascular interaction is an important step leading to a breach in the microvascular barrier can also be seen in experiments using monoclonal antibodies against the neutrophil adhesion receptor complex (CD 18). This manipulation prevents neutrophil-endothelial adhesion and attenuates the vascular leak following ischemia or shock (12,31). This present study was designed to test more directly the role of the activated neutrophil following

localized aspiration in inducing multi-organ injury.

METHODS

Animal Preparation

Eighty-four adult male Sprague-Dawley (Charles River Lab., Wilmington, MA) weighing approximately 500 g were anesthetized with intraperitoneal ketamine (35 mg/kg). A jugular venous catheter was inserted for fluid or drug infusion (1 ml/hour) and hourly intravenous anesthetic dosing (ketamine, 8 mg/kg; xylazine 1 mg/kg). A tracheostomy was performed with a 15-gauge tube. Through this tube, a fine-bore polyester cannula with an external diameter of 0.61 mm and an internal diameter of 0.28 mm was introduced into the anterior segment of the left lung which represented approximately one-third the weight of the left lung. All animals were supine for the duration of the experiment.

Preparation of Solutions[1]

Hydrochloric acid: 0.1 ml 33% HCl (McGaw Park, IL) was mixed with 9 ml of 0.9% NaCl. The final concentration was 0.1 N. When aspirated in a volume of 0.1 ml the mortality rate within 3 hours was less than 10%. A lower concentration of 0.05 N HCl did not lead to systemic effects, whereas increased concentrations of greater than 0.2 N or volumes of greater than 0.2 ml increased mortality rates above 30%.

Nitrogen Mustard: Mechlorethamine (2-chloro-N-[2-chloroethyl]-N- methylethanamine) was diluted in saline to a final concentration of 0.75 mg/kg. To induce leukopenia the drug was

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1 Unless otherwise noted, chemicals and reagents were obtained from Sigma Chemical Co., St. Louis, MO.

infused via a jugular venous catheter 64 hours prior to the experiment. The catheter was then removed. A new catheter was inserted on the opposite side on the day of the experiment, at which time the circulating PMN count was $140 \pm 20/mm^3$ (Table 1). The animals were kept in isolation. Nitrogen mustard is known to be inactivated within minutes. Further, the dosage used in these experiments was thought not to affect lung or systemic endothelium (20) in that animals showed no alteration in organ weights.

Anti Neutrophil Serum : Leukopenia was accomplished in another experimental group by using rabbit antiserum to rat neutrophils (26). Neutrophils were obtained by an intraperitoneal instillation of 1% glycogen, followed by peritoneal lavage with phosphate-buffered saline (PBS) 4 hours later. After centrifugation and resuspension, the few contaminating erythrocytes were removed by hypotonic lysis using half strength PBS for 30 seconds. Rabbits were innoculated intra-dermally with 40×10^6 rat neutrophils suspended in 1 ml of complete Freund's adjuvant. An identical challenge was administered 3 weeks later. The rabbits were bled after another 7-10 days and the sera pooled and frozen. The rabbit antiserum was injected intraperitoneally at 0.5 ml/100 g body weight, 18 hours before the experiment.

Flow Cytometry

Intracellular generation of H_2O_2 by blood neutrophils was quantitated using flow cytometry and dichlorofluorescein-diacetate (DCFH). DCFH is a non-fluorescent compound which is oxidized to the highly fluorescent dichlorofluorescein (DCF) within neutrophils undergoing a respiratory burst. Leukocytes were isolated from blood using dextran sedimentation, 6% in 0.9% saline, (0.3 ml dextran solution per 3 ml blood) for 45 minutes at room temperature (Dextran T 500, Pharmacia, Piscataway, NJ). In preliminary experiments, it was found that any centrifuga-

tion, vortexing or even vigorous pipetting led to increases in baseline oxidation in neutrophils, so these procedures were eliminated in the final protocol. Aliquots of leukocyte rich sediment (0.01 ml) were added to 1 ml of balanced salt solution (BSS). This solution contained NaCl (124 mM), KCl (5.8 mM), dextrose (10mM) and hydroxyethylpiperazine ethanesulphonic acid (20 mM) and was titrated with NaOH to pH 7.4 prior to use. The BSS also contained DCFH (Molecular Probes, Eugene, OR) and either buffer or phorbol myristate acetate (PMA) 10^{-7}M. The concentration of DCFH was 100μM, an amount which saturated leukocytes in samples from sham or experimental animals. After incubation for 20 minutes at 37°C the samples were placed on ice and subsequently analyzed with an Ortho Diagnostic System 2151 Cytofluorograph flow cytometer using the 488 ηm excitation line of an argon laser at 125 mw output. The PMN within each sample were identified by light scattering. After electronic gating, the green fluorescence of these cells in unstimulated and PMA stimulated samples was quantitated (3,000-5,000 neutrophils per sample). In some experiments, the fluorescence values obtained by flow cytometry were calibrated with samples of pure rat neutrophils. These cells were obtained 4 hours after intraperitoneal glycogen. The cells were suspended in BSS, 10^6 cells/ml and labelled with DCFH with or without PMA treatment. Measurements were conducted in a fluorometer (Perkin-Elmer, Norwalk, CT). Using a standard curve constructed with reagent grade DCF (Sigma Chemical Co.), the amount of DCFH oxidized to DCF by neutrophil H_2O_2 was quantitated, allowing conversion of the mean fluorescence channel number to fentamoles (fm) DCF produced per cell, a value approximately equivalent to fm of H_2O_2 produced per cell (3).

Eicosanoid Assay

Concentration of TxB_2 in plasma was measured in duplicate with a double radioimmunoas-

say, using an antibody whose cross-reactivity with heterologous prostanoids was less than 1%. Leukotriene (LT)B_4 was also measured in duplicate by radioimmunoassay (rabbit antibody and standards were obtained from Seragen, Cambridge, MA). Cross-reactivity of the LTB_4 antibody with other LT's, TxB_2, the prostaglandins and their metabolites was also less than 1%.

Myeloperoxidase (MPO)

Activity of this neutrophil enzyme was used as a tracer to quantitate PMN sequestration in systemic tissue where it was found to be a more sensitive assay for the detection of entrapped neutrophils than histology. It was assayed immediately upon removal of tissue specimens (10,19). One gram of tissue blotted dry was homogenized in 10 ml of 0.01 M potassium phosphate buffer (PPB, pH 7.4) containing ethylene diamine tetracetic acid (EDTA). Two ml of homogenate and 5 ml of 0.01 M PPB containing 1.0 mM EDTA were gently mixed and then centrifuged at 10,000 x g for 20 minutes at 4°C (PR-2 centrifuge, International Equipment Company, Boston, MA). The pellet was re-homogenized in 5 ml of 0.05 M PPB (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HTAB). The pellet-HTAB suspension was freeze-thawed and sonicated with a Branson cell disrupter (Heat Systems, Ultronics, Plainview, NY) at 65 watts for 1 minutes. A 0.1 ml aliquot was mixed with 0.79 ml of 0.08 M PPB (pH 5.4) and 0.1 ml of 16 mM tetramethylbenzidine dissolved in N,N-dimethylformamide at 37°C. After 2 minutes, 0.01 ml of 30 mM H_2O_2 was added. After incubating 3 minutes at 37°C, 0.05 ml of catalase solution, 300 μg/ml, was added. The mixture was diluted with 4 ml of 0.2 M sodium acetate (pH 3.0) and then centrifuged at 12,000 x g for 10 minutes at 4°C. The supernatant was read in a spectrophotometer (Spectronic 601, Milton Roy, Rochester, NY). One unit of MPO activity was arbitrarily defined as the amount of enzyme necessary to catalaze an increase

in absorbance of 1.0 at 655 nm per minute at 37°C.

White Cell Count

Circulating white cells and platelets were quantitated by phase microscopy. Differential counts were made on Wright's stained blood smears.

Experimental Protocol

Evans Blue dye 0.2 mg was added to solutions used for aspiration for later confirmation of the site of introduction. Four groups were studied. Three were pretreated with IV saline (n=24), or nitrogen mustard (n=18), or anti neutrophil serum (n=18) and then underwent localized aspiration of 0.1 ml 0.1N HCl. The last group (n=24) was pretreated with IV saline and then underwent aspiration with 0.1 ml of 0.9% saline.

The intrabronchial cannula was taken out after aspiration. Three hours later the animal was euthanased with an overdose of 200 mg ketamine. A thoracotomy was performed and the right and left lung bronchi were clamped in turn. Bronchoalveolar lavages of the left lung, including the aspirated segment and the right lung were then performed in sequence. For each lung, 3 ml of saline was lavaged using the tracheostomy tube. This was repeated three times. The combined lavage return of about 8 ml was introduced into tubes containing 0.3 ml of 0.07M EDTA. This BAL fluid was centrifuged at 1500 x g for 20 minutes (GLC-1 Centrifuge, Sorvall, Newtown, CT) and frozen at -20°C and subsequently used for assay of protein concentration using the spectrophotometric protein dye method (6). Six rats from each experimental group were used for lavage. Another six were used to calculate the wet to dry weight (W/d) ratios of the aspirated and non-aspirated lung segments. This was done after weighing the dyed segment of freshly harvested lung tissue, heating the segment at 90°C in a gravity convection oven (Pre-

cision Scientific Group, Chicago, IL) to constant weight for 72 h, and weighing the residuum. A similar method was used to measure the W/d ratio of the heart and kidney. Six animals from each group were used for lung histology. After euthanasia the lungs were perfused with 10% formaldehyde and then inflated with the same material to a pressure of 25 cm H_2O. Following fixation, sections from dyed and contralateral segments were taken and stained with hematoxylin and eosin for light microscopic analysis. All microscopic sections were interpreted in a blind fashion by a pulmonary pathologist (LK). Lung sequestration of PMN's was quantitated by counting alveolar septal wall PMN's in the dyed segment and in the contralateral segment. Only peripheral lung parenchyma was examined. Microscopic fields containing other structures such as airway, large vessels, and pleura were excluded. Leukocyte entrapment was expressed as the mean number of PMN per ten high power fields (x 1000). Animals in which Evans Blue dye appeared in the non-aspirated lung were excluded.

Results are presented as mean ± SEM in text, Table and Figures. Statistical analysis was conducted by last square regression and by an analysis of variance. In the latter case if significance was demonstrated, further evaluation was done by a non paired Student's t-test. Significance was accepted if $p < 0.05$.

Animals in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (Department of Health, Education and Welfare, Publication No. 78-23 (National Institute of Health), revised, 1978.

RESULTS

Thirty minutes following local HCl aspiration, intracellular H_2O_2 production of circulating PMN increased to 20.3 ± 5 fmDCF/PMN (n=6) relative to 2.7 ± 0.4 fmDCF/PMN in saline aspirated control animals (n=6, $p < 0.05$, Fig. 1). Incubation of additional aliquots of these PMN with PMA 10^{-7}M led to an enhanced oxidative response to 132 ± 15 fmDCF/PMN following acid aspiration relative to the value of 49.5 ± 15 fmDCF/PMN in control animals ($p < 0.05$). This event was short-lived. Thus, after 45 min H_2O_2 production returned to baseline levels 6.2 ± 4 and with PMA stimulation to 52 ± 20 fmDCF/PMN a value similar to control. After 60 min the response to PMA was further reduced to 18 ± 3 fmDCF/PMN.

After 30 min, acid aspiration led to increases in plasma concentration of TxB_2 1340 ± 215 pg/ml higher than control animals ($p < 0.05$). At 3 hours Tx levels remained elevated (Fig. 2). At 30 minutes, PMN sequestration in the non-aspirated lung was first noted (Fig. 3) and there was leukopenia to 2650 ± 300 WBC/mm^3 relative to control values of 10,500 ± 570 WBC/mm^3($p < 0.05$).

Lung neutrophil sequestration in the non-aspirated side was progressive over the 3 h period of monitoring. MPO activity of the heart and kidney rose at 30 minutes, appeared to plateau before rising again at 2 hours, a curve similar to the non-aspirated lung. The secondary rise in MPO in heart and kidney was temporally related to the increase in W/d weight of these organs (Fig. 4,5). The non-aspirated lung also was noted to follow this delayed course of W/d increase (Fig. 5) despite early lung leukosequestration (Fig. 3). Further, after 3 hours PMN sequestration in the non-aspirated lung and W/d weights were significantly correlated (Fig. 6).

At 3 hours neutrophil sequestration was demonstrated in both aspirated and non-aspirated lung segments, values being 70 ± 5 PMN/10HPF and 51 ± 4 PMN/10HPF respectively compared to control rats with 7 ± 4 PMN/10HPF in the aspirated and 5 ± 6 PMN/10HPF in the non-aspirated segments (both $p < 0.05$). Protein concentration in BAL fluid was 3330 ± 180 μg/ml and 2540 ± 160 μg/ml in the aspirated and non-aspirated regions. These values were higher than in control animals 482 ± 62 and 411+120 μg/ml respectively (all $p < 0.05$)(Fig. 7).

At 3 hours animals made leukopenic with nitrogen mustard (Table 1) showed attenuation of the acid aspiration induced plasma TxB_2 and LTB_4 generation to 540 ± 107 and 240 ± 20 pg/ml respectively (Fig 2, $p < 0.05$). As expected leukopenia using either technique prevented lung neutrophil sequestration as well as the rise in MPO activity of the heart and kidney (Fig. 8). Leukopenia led to a reduction in protein leak in both aspirated and non-aspirated segments to 2370 ± 85 and 1690 ± 180μg/ml with nitrogen mustard and to 2460 ± 180μg/ml and to 800 ± 190μg/ml with anti neutrophil serum (all $p < 0.05$) (Fig. 7). It also prevented the increase in W/d weight ratio of the non-aspirated lung and attenuated the increase of the aspirated side ($p < 0.05$) (Fig. 9). Finally, the increase in the W/d weights of the heart and kidney were prevented ($p < 0.05$, Fig. 10). Control leukopenic rats (n=4) treated with nitrogen mustard, 3 hours following saline aspiration showed no difference in W/d ratios of the lungs (3.7 ± 0.2 and 3.5 ± 0.2), heart (3.5 ± 0.2) or kidney (3.6 ± 0.3) relative to control rats.

DISCUSSION

The data of this study indicate that localized acid aspiration leads to activation of circulating neutrophils, and that their later sequestration in the microcirculation of the lungs and sys-

temic organs is coincident with the development of edema in these organs. That neutrophils cause edema is shown by: 1. The temporal relationship of the late lung neutrophil sequestration, after 2 hours and the late MPO rise in the heart and kidney with increase in the W/d weight ratios of these organs (Fig. 3-5); 2. A correlation at 3 hours between the number of neutrophils sequestered in the non-aspirated lung and the W/d weight ratio (Fig. 6); 3. The attenuation of permeability increase in the lungs and the prevention of W/d weight gain in lungs and systemic organs in leukopenic animals (Fig. 7,9,10).

Thirty minutes after aspiration there was an increase in oxidative activity of circulating neutrophils. These cells were also primed for a second oxidative stimulus. In another study of acid aspiration, thromboxane was found to mediate this neutrophil oxidative activity. Indeed, inhibition of Tx synthesis with OKY 046 or Tx receptors with SQ 29,548 prevented the acid induced neutrophil oxidative burst as well as the enhanced oxidative response to phorbol ester (8). A previous study has shown that the early Tx synthesis, 30 minutes following aspiration is from circulating platelets (30). We postulate that this early, high level of circulating TxA_2 stimulates PMN H_2O_2 synthesis. Thromboxane A_2 as well as LTB_4 may also upregulate the surface leukocyte CD18 adhesion receptor, a phenomenon which is independent of oxidative activity but which may explain the significant leukopenia as well as the early lung leukosequestration (1). This rapid lung neutrophil sequestration can be induced experimentally. Thus, 30 minutes after intra-arterial or intravenous infusion of zymosan activated plasma there is an upregulation of PMN CD 18 adhesion receptors and increased sequestration of PMN in the lungs (17,14). In unpublished studies we have noted this occurrence also with LTB_4 infusions. In both these studies the neutrophil sequestration appeared to be selective in the lungs. This was not the case 30

minutes following aspiration where the rise in PMN/10HPF was 2.5 fold in the non-aspirated lung and the rise in MPO was about 3 fold in the heart and kidney (Fig. 3,4). There is insufficient data to resolve these discrepant observations. It is possible that a different chemoattractant other than complement or LTB_4 was operative in the present study of aspiration. This multi-organ inflammatory response following acid aspiration has been previosly reported (22). Thus, dogs that were subjected to localized HCl aspiration showed a generalized increase in permeability.

The oxidative activity of circulating PMN appeared to be a short-lived event. Thus, after 45 minutes, H_2O_2 production returned to baseline levels and at 60 minutes there was tachyphylaxis to a second stimulus. Further, the intracelluar production of H_2O_2 was not temporally related to lung or systemic organ edema. This is possibly explained by the fact that neutrophils appear to require interaction of CD 18 with endothelial ligand(s) in order to release their intracellular H_2O_2 (23,24). The later, 2 hour, adhesive process of PMN in the lungs, heart and kidney occurs at a time consistent with upregulation of new endothelial adhesion receptors such as intercellular adhesion molecule-1 (ICAM-1) and the endothelial leukocyte adhesion molecule-1 (ELAM-1). Further, it is also at a time when PMN have been shown to become metabolically active in the synthesis of TxA_2 (30). It is at this point that the lungs, heart and kidney start becoming edematous and the increase in W/d is related to leukosequestration (Fig. 3,4,5,6). The stimulus to PMN release of Tx and possibly H_2O_2 could be their interaction with newly expressed ICAM-1 or ELAM-1 (4,27). In addition to eicosanoids and H_2O_2, other PMN contents such as elastase may also be released and mediate permeability.

In vitro studies show the upregulation of ICAM-1 and ELAM-1 to occur over 2 to 3 hours

and to be dependent upon the action of a cytokine such as tumor necrosis factor (TNF) or interleukin-1 (IL-1) (5,7). Our group has shown that TNF-α antiserum will, in fact, prevent leukosequestration following aspiration in both the aspirated and non-aspirated lung as well as in the heart and kidney (9). That this delayed leukosequestration is related to newly synthesized endothelial adhesion proteins is further supported by observations that the protein synthesis inhibitor, cycloheximide is able to block PMN accumulations in the lungs and systemic organs (9).

Neutrophils have been found to mediate injury in other experimental models of the adult respiratory distress syndrome (ARDS). Thus, neutrophil depletion prevented the lung injury following remote ischemia or hypovolemic shock (18,2). In the present study, severe neutropenia largely but not completely, prevented acid induced lung and systemic organ injury. Protection was least effective in the aspirated side of the lung (Fig. 7,9). This is not surprising, since the direct local effect of HCl in denaturing protein and increasing permeability would not be reversed by neutropenia. Indeed, others have reported that accumulation of protein in BAL fluid precedes neutrophil sequestration in the HCl lavage segment (15). Further, the partial protection may be consistent with the failure to induce complete neutropenia as well as the failure to remove large numbers of lymphocytes and monocytes (Table 1). Finally, it is also possible that other mediators are involved. This latter possibility has been documented in studies of severely leukopenic patients who still manifest ARDS (25).

Other non inflammatory mechanisms might augment the organ edema. Thus, pulmonary hypertension is known to accompany acid aspiration and to accentuate the edema in the presence of an injured pulmonary microvascular membrane (13). In addition, secondary right ventricular failure is possible and this could influence the rise in W/d weight of the heart and kidney.

In summary, localized acid aspiration leads to activation of circulating neutrophils and promotes their sequestration in the lungs and systemic organs. These cells mediate in large part the increase in permeability and edema of these organs.

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TABLE 1

EFFECTS OF NITROGEN MUSTARD AND ANTI-NEUTROPHIL SERUM ON WBC AND PLATELET COUNTS (Cells/mm^3)

Treatment	WBC	PMN	Lymphocyte	Monocyte	Platelets
Saline	7150 ± 550	1480 ± 140	5300 ± 410	350 ± 60	340±50x10^3
Nitrogen Mustard	1780 ± 280*	140 ± 20*	1750 ± 240*	140 ± 20*	400±70x10^3
Anti-Neutrophil Serum	4200 ± 170*	150 ± 20*	3840 ± 200*	220 ± 20*	380±60x10^3

* Indicates $p < 0.05$ relative to saline treatment values.

Figure 1. After 30 min acid aspiration induced a neutrophil oxidative burst and also primed the cells to a second stimulus (PMA). After 45 min oxidative activity return to baseline and after 60 min tachyphylaxis to PMA was noted . The symbols * and † indicate $p < 0.05$ relative to background and control values respectively.

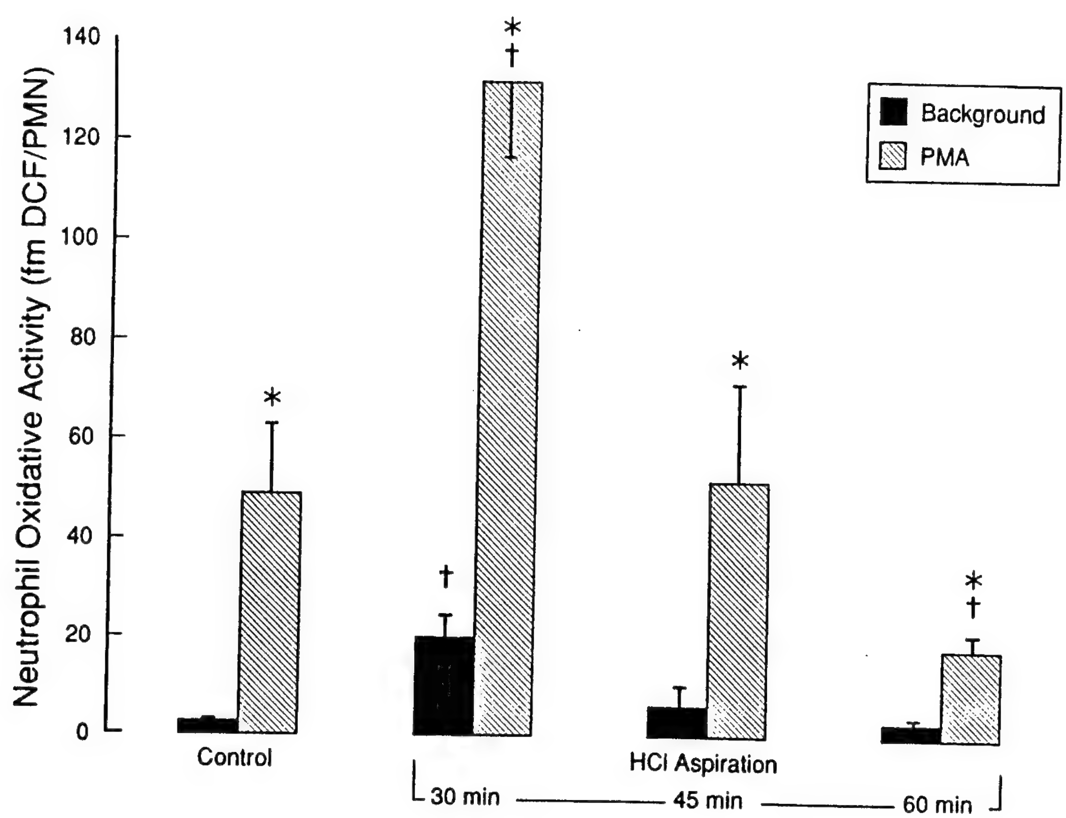

Figure 2. Three hours after localized acid aspiration eicosanoid levels were increased, an event attenuated in animals rendered neutropenic. The symbols * and † indicate $p < 0.05$ relative to saline and neutropenic animals respectively.

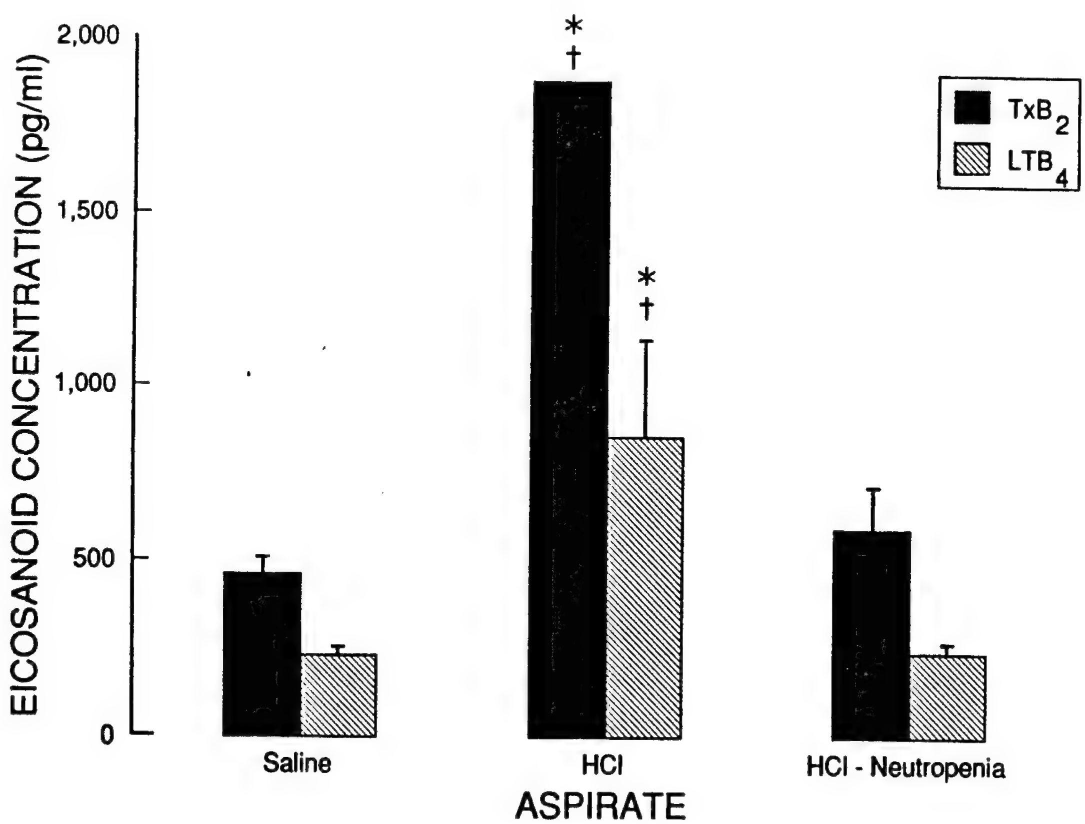

Figure 3. Kinetics of neutrophil sequestration in the non-aspirated lung show a significant ($p < 0.05$) increase in numbers 30 minutes after aspiration at a time that leukopenia was noted. Thereafter PMN sequestration was progressive.

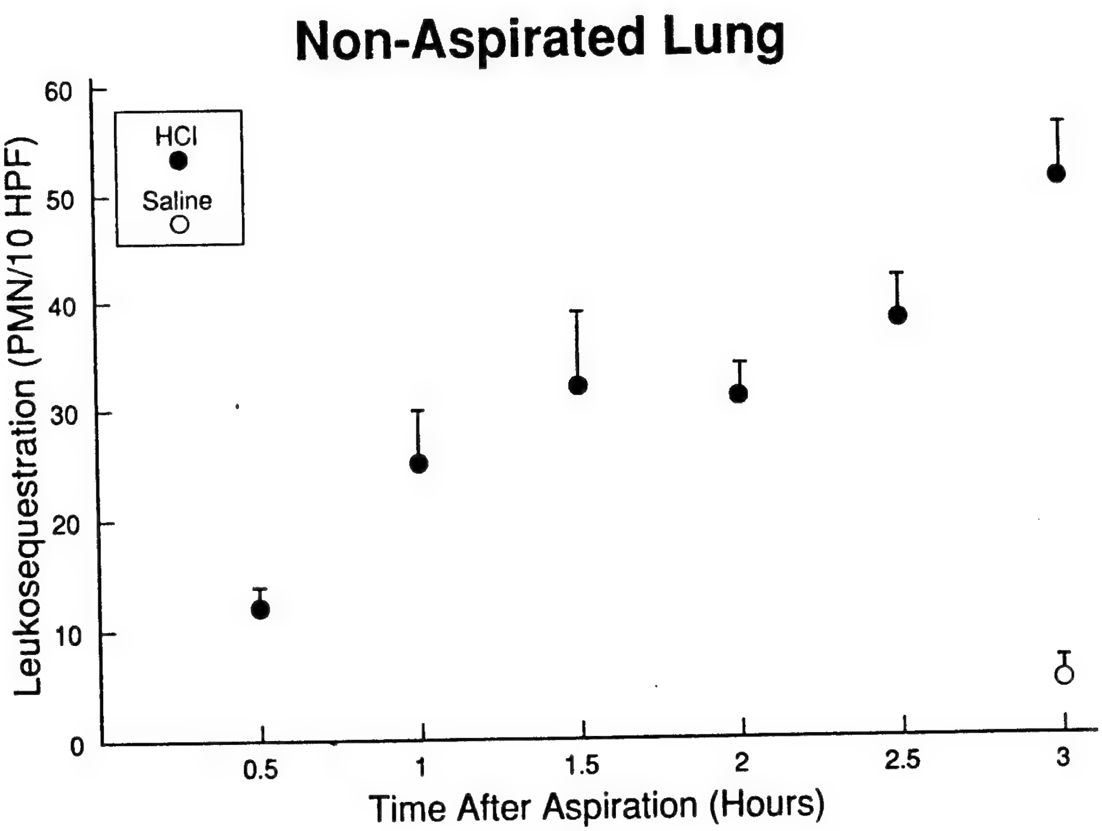

Figure 4. Kinetics of increased myeloperoxidase activity in systemic organs demonstrate that 30 minutes after aspiration there was a small but significant rise relative to saline aspirated animals ($p < 0.05$). After 2 hours another rise in MPO was noted.

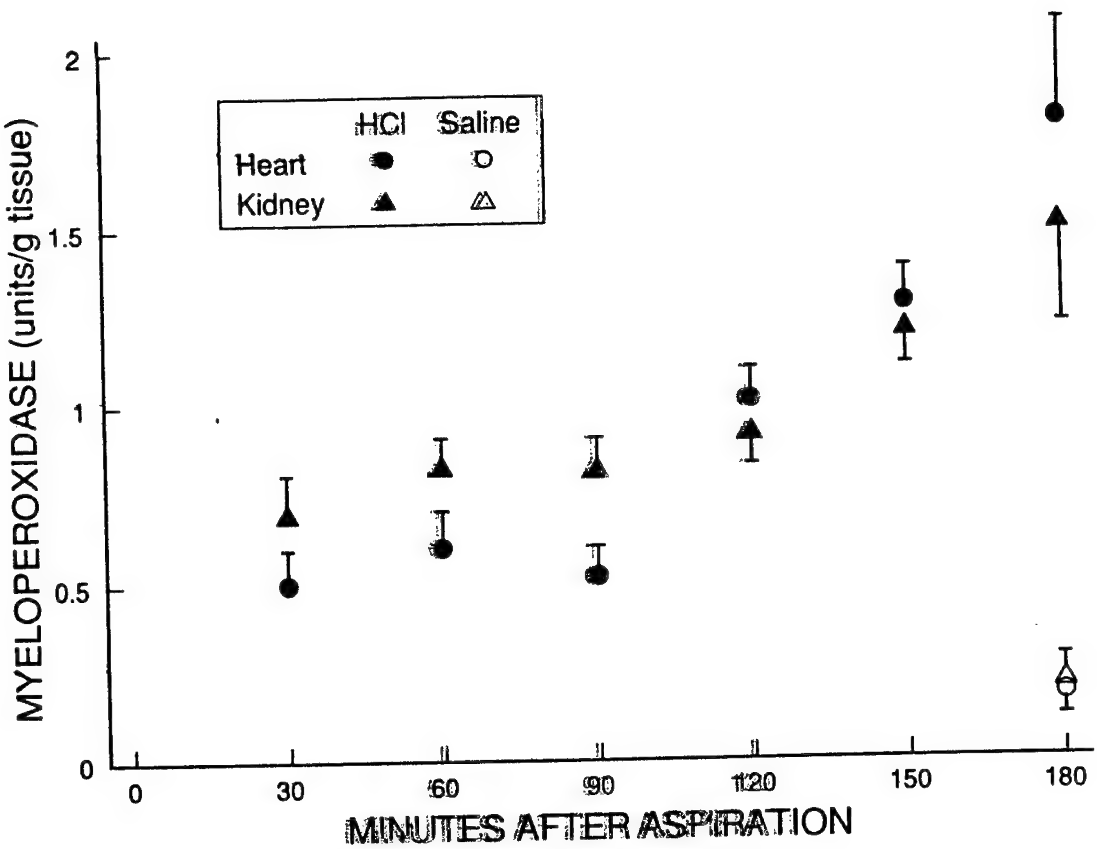

Figure 5. Edema of the non aspirated lung, heart and kidney was noted 2 to 2.5 hours following aspiration at the time neutrophil sequestration became prominent (Fig. 4) Leukopenia was protective.

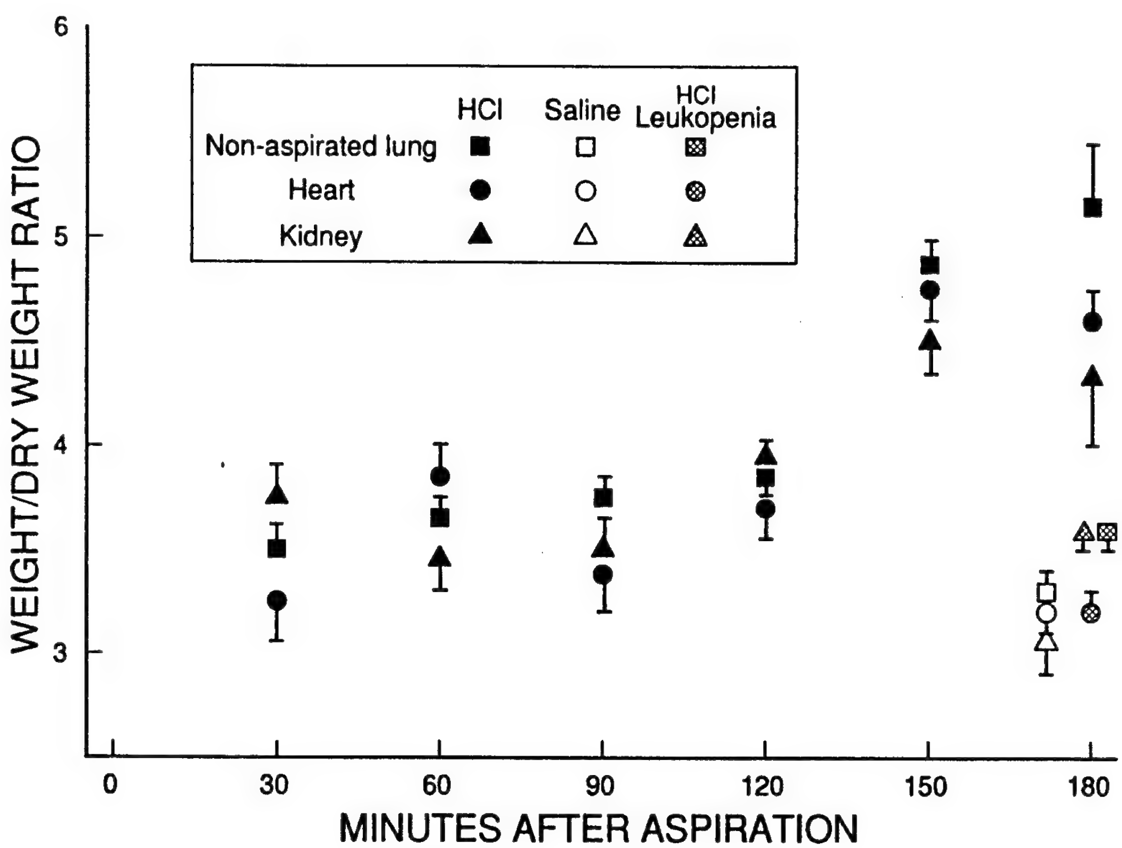

Figure 6. At 3 hours a significant correlation ($p < 0.05$) was found between neutrophil sequestration in the non-aspirated lung and the increase in W/d weight ratio.

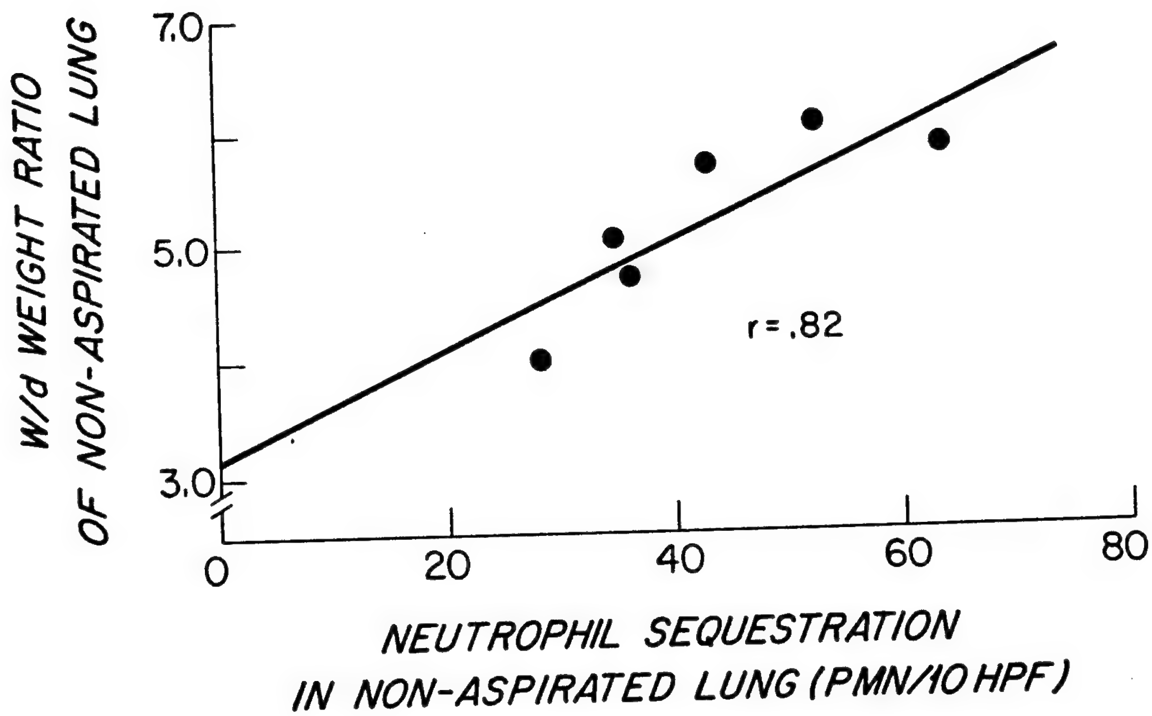

Figure 7. Localized acid aspiration induced a generalized increase in pulmonary permeability as shown by protein leakage into BAL fluid of the aspirated and non-aspirated lungs. The symbols * and † indicate < 0.05 relative to saline and neutropenic animals respectively.

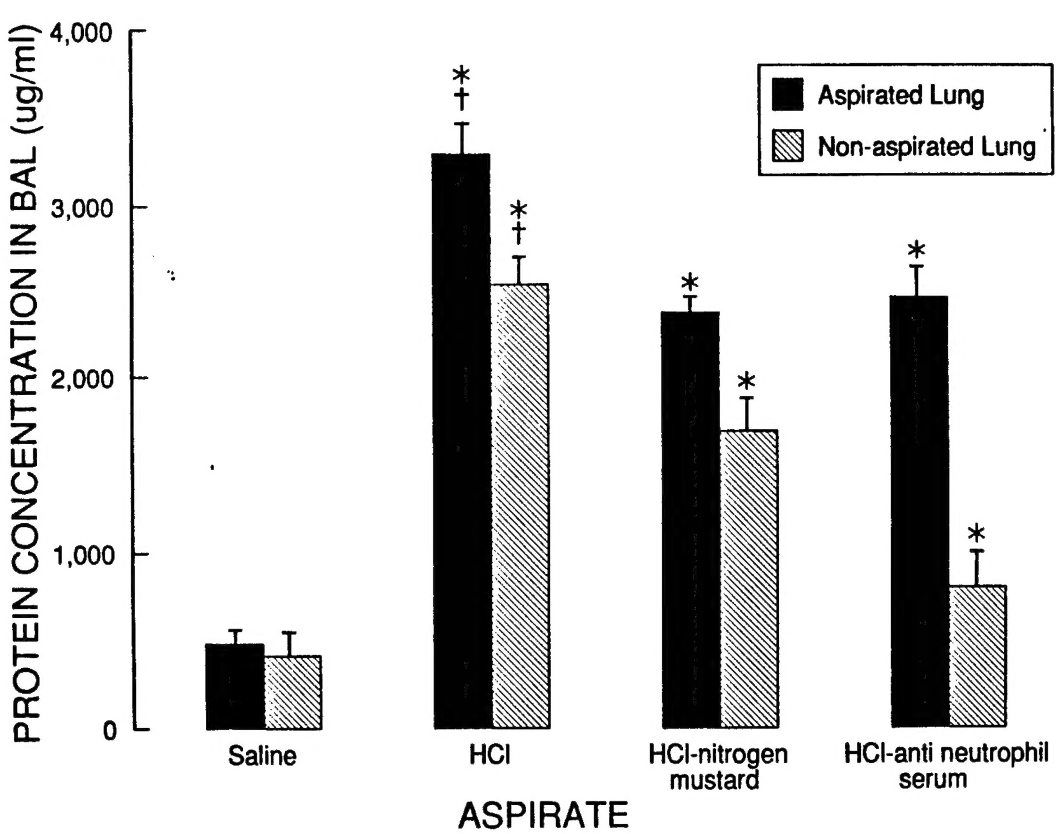

Figure 8. After 3 hours, localized acid aspiration led to increased MPO activity in systemic organs. The symbols * and † indicate $p < 0.05$ relative to saline and neutropenic groups respectively.

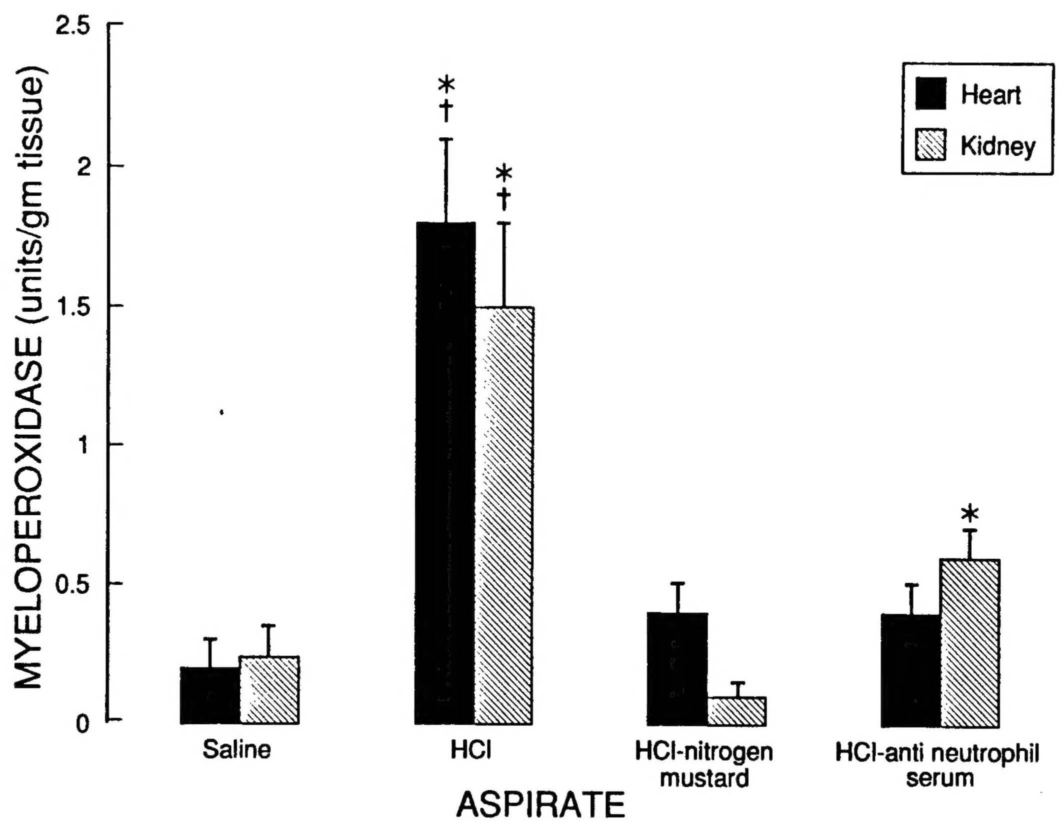

Figure 9. Localized acid aspiration induced generalized lung edema after 3 hours. Neutropenia attenuated this event on the aspirated side and fully prevented it on the non-aspirated side. The symbols * and † indicate $p < 0.05$ relative to saline and neutropenic animals respectively.

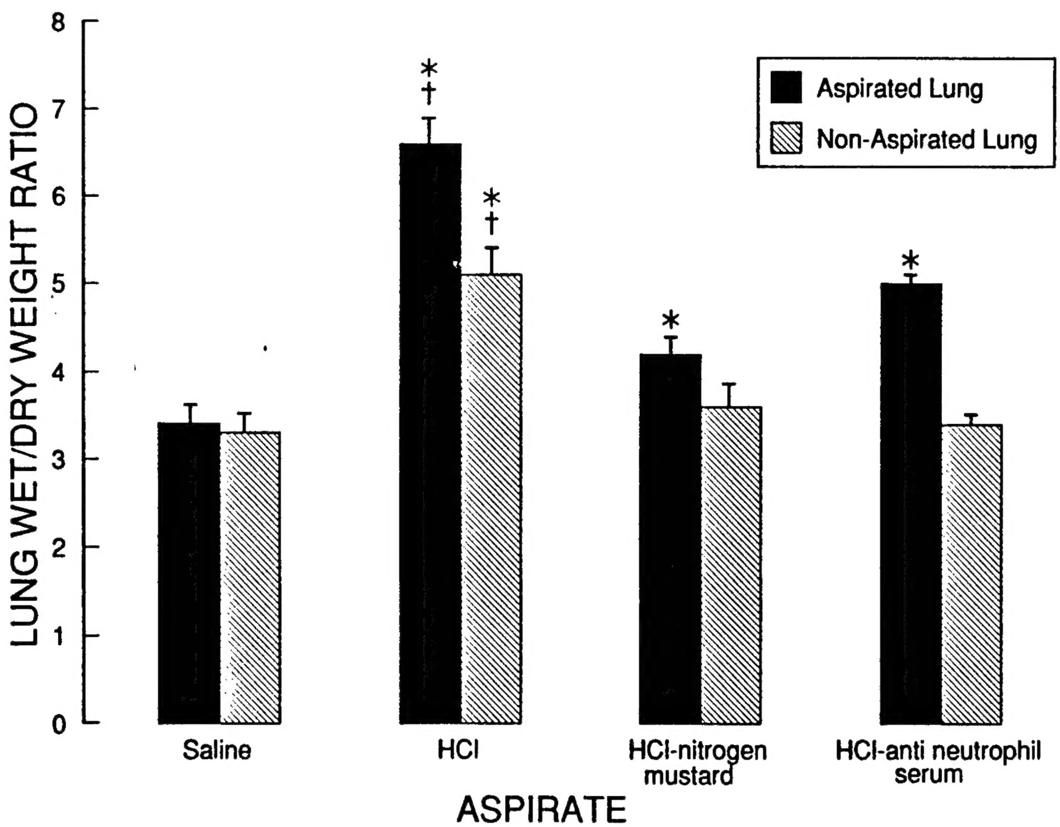

Figure 10. Localized aspiration induced systemic organ edema after 3 hours. Neutropenia prevented this event. No significant difference was noted between neutropenic and saline control animals. The symbols * and † indicate $p < 0.05$ relative to saline and neutropenic animals.